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(71) Applicant (*for all designated States except US*): INTERFACE MULTIGRAD TECHNOLOGY (IMT) LTD. [IL/IL]; Hamamah Technologit Yuzmot Haemek, 23100 Migdal Haemek (IL).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): ARAV, Amir [IL/IL]; Shlomzion Hamalkah 59, 62266 Tel Aviv (IL).

(74) Agent: FRIEDMAN, Mark, M.; c/o Castorina, Anthony, Suite 207, 2001 Jefferson Davis Highway, Arlington, VA 22202 (US).

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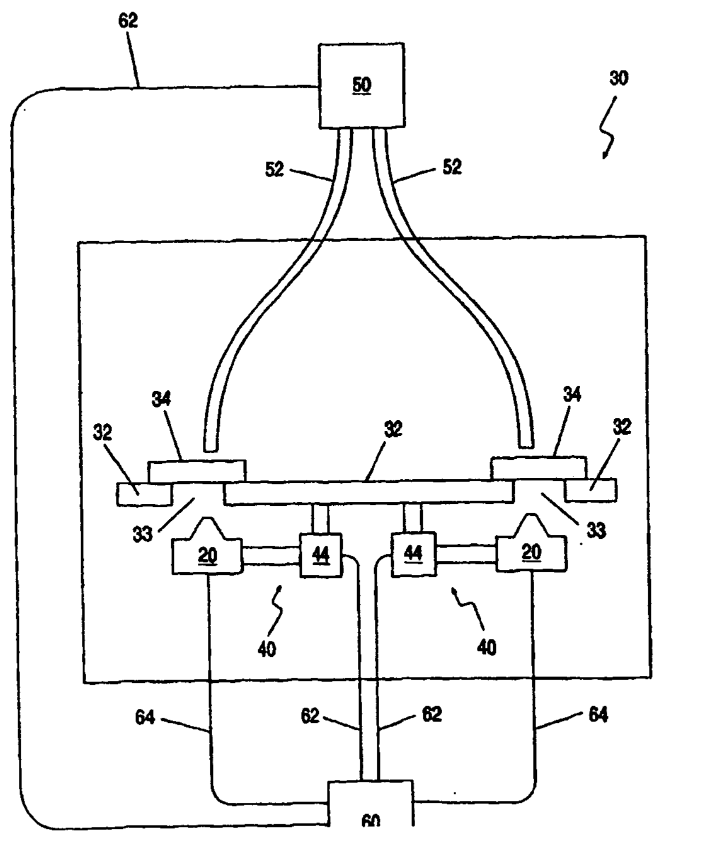
(54) Title: METHOD AND APPARATUS FOR MONITORING A BIOLOGICAL SAMPLE

(57) Abstract

A method and apparatus for monitoring biological samples (34) comprise an incubator (30) within which are mounted at least two CCD mini-cameras (20) adapted for photomicroscopy as mini-photomicroscopes. The apparatus may be configured for time-lapse photomicroscopy, transmission photomicroscopy, reflection photomicroscopy, epifluorescence photomicroscopy, or infrared photomicroscopy. Three-dimensional images are acquired by focusing the mini-photomicroscopes on successive focal image planes in the biological samples (34). The mini-photomicroscopes may be focused on separate samples, on different portions of the same sample, or on the same portion of the same sample.



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METHOD AND APPARATUS FOR MONITORING A BIOLOGICAL SAMPLE

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a method for monitoring the development of living biological samples and, more particularly, to an apparatus and method for monitoring the development of incubating cell cultures such as embryos.

Cell cultures commonly are grown inside incubators. An incubator is a closed box within which the environmental parameters, such as temperature, humidity and atmospheric composition, can be optimized to promote the growth of the cell cultures. For example, mammalian embryos should be incubated under conditions resembling those found inside a mammalian womb.

It is advantageous to monitor the growth of cell cultures using a microscope, so that the details of the development of individual cells may be observed. If a photomicroscope is used, it can be focused on successive focal image planes within the culture, in a manner similar to that taught by Carlsson in US Patent No. 4,631,581 for microphotometry of prepared biological specimens, to record successive two-dimensional slices through the cell culture, thereby obtaining a three-dimensional record of the structure of the cell culture. Because microscopes are large, bulky, delicate instruments that do not fit inside commonly used incubators, it has been the practice heretofore to enclose microscope stages in specially constructed incubators so that those microscopes could be used to monitor incubating cells. This clearly is an awkward procedure. Furthermore, this procedure allows only one cell culture, or only one portion of a cell culture, to be monitored within the incubator at any given time.

This problem is addressed partially by Miyamoto in US Patent No. 5,307,161. Miyamoto places a solid-state area image sensor array, such as a charge coupled device (CCD) array, in close proximity to a biological sample within an incubator. CCD arrays are small enough to fit inside commonly used incubators. If positioned close enough to the biological sample, a CCD array does not need an optical system in order to image the sample. Several biological samples may be monitored simultaneously, each by its own CCD array. Signals from the CCD array are transmitted to a display unit such as a video monitor, and also may be digitized, processed and stored in the conventional manner. The drawback of using a solid-state area image sensor array without an optical system, as taught by Miyamoto, is that the resolution of the images obtained is limited by the size of the sensor elements that comprise the array. Sample features smaller than the width of one sensor element cannot be imaged.

There is thus a widely recognized need for, and it would be highly advantageous to have, a method for microscopic monitoring of a living cell culture within a conventional incubator.

SUMMARY OF THE INVENTION

According to the present invention there is provided a method for monitoring the development of a biological sample in an incubator, comprising the steps of: (a) providing, inside the incubator, at least two mini-microscopes, each of the mini-microscopes including: (i) a microscope objective, and (ii) a solid-state area image sensor array, optically coupled to the microscope objective; (b) placing the biological

sample in a transparent holder inside the incubator; and (c) focusing each of the at least two mini-microscopes on a focal image plane, at a focal distance from the mini-microscope, in the biological sample.

According to the present invention there is provided an apparatus for monitoring the development of a biological sample, comprising: (a) an incubator; (b) a transparent holder mounted within the incubator; and (c) at least two mini-microscopes, mounted relative to the transparent holder within the incubator so that, when the biological sample is placed in the transparent holder, each of the at least two mini-microscopes may be focused on a focal image plane within the biological sample.

The present invention is made possible by a newly available type of camera, that uses a CCD array instead of photographic film as its light-sensitive element. Such "CCD mini-cameras" are manufactured, for example, by Aplitec Ltd. of Holon, Israel. These cameras are small enough to fit comfortably inside conventional incubators. Figure 1A is a schematic cross section through a CCD mini-camera **10**, showing the parts of the camera that are relevant to the present invention. A housing **12** is provided with an optical system **14** for focusing light on a CCD array **16** enclosed by housing **12**. Optical system **14** may be removed and replaced by a different optical system, depending on the use to which camera **10** is to be put. In particular, optical system may be replaced, as shown in Figure 1B, by an adapter **22** and a conventional microscope objective **24**. Adapter **22** is configured to position objective **24** at a distance from CCD array **16** at which a magnified image of an object immediately in front of objective **24** is focused on CCD array **16**. This converts CCD

mini-camera **10** into a CCD mini-photomicroscope **20**. For reference below, the optical axis of mini-photomicroscope **20** is designated by the reference numeral **26**. Although mini-photomicroscope **20** is shown configured with CCD array **16**, the scope of the present invention includes mini-photomicroscopes configured with any suitable solid-state area image sensor array.

Mini-photomicroscope **20** is small enough so that several mini-photomicroscopes **20** may be mounted inside a conventional incubator. The various configurations in which mini-photomicroscopes **20** may be mounted within an incubator, and used to monitor the development of biological samples such as cell cultures, are discussed below.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1A (prior art) is a schematic cross-section of a CCD mini-camera.

FIG. 1B is a schematic cross-section of the mini-camera of FIG. 1A reconfigured as a mini-photomicroscope.

FIG. 2 is a schematic diagram of an incubator system provided with two of the mini-photomicroscopes of FIG. 1B, for monitoring biological samples in Petri dishes.

FIG. 3 is a schematic diagram of two of the mini-photomicroscopes of FIG. 1B, deployed at right angles to monitor a biological sample in a square capillary tube.

FIG. 4 is an alternative configuration of the mini-photomicroscope of FIG. 1B.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of a method and apparatus for monitoring the development of incubated biological samples. Specifically, the present invention can be used to monitor the development of incubated cell cultures such as embryos.

The principles and operation of incubator miniphotomicroscopy according to the present invention may be better understood with reference to the drawings and the accompanying description.

Referring now to the drawings, Figure 2 is a schematic diagram of one embodiment of an apparatus for monitoring the development of a biological sample, according to the present invention. The apparatus comprises an incubator **30**, within which transparent holders for biological samples, in this case two Petri dishes **34**, are placed above holes **33** in a shelf **32**. Below Petri dishes **34** are two mini-photomicroscopes **20**, mounted on mountings **40**. Mountings **40** include step motors **44** that move mini-photomicroscopes **20** up and down to focus, from different focal distances, on different focal imaging planes within the biological samples in Petri dishes **34**. For simplicity, Figure 2 shows only two mini-photomicroscopes **20**; the preferred embodiment of the apparatus includes at least four mini-photomicroscopes. Furthermore, although Figure 2 shows each of mini-photomicroscopes **20** mounted above a different Petri dish **34**, the scope of the present invention includes configurations in which several mini-photomicroscopes are mounted below the same transparent holder, with each mini-photomicroscope monitoring the development of a different biological sample in the transparent holder, or, alternatively, with each mini-photomicroscope monitoring a different portion of the same biological sample.

Light from a light source **50** is directed at Petri dishes **34** from above by optic fiber cables **52**, so that mini-photomicroscopes **20** capture images of the biological samples in Petri dishes **34** by transmitted light. Light source **50** and stepping motors **44** are controlled by a control system **60**, which is connected to light source **50** and stepping motors **44** by suitable electrical connections **62**, such as coaxial cables. Similar connections **64** are used to convey signals from the solid-state area image sensor arrays of mini-photomicroscopes **20** to control system **60**. The signals may be digitized, and the corresponding digital images may be displayed on a monitor equipped with an image splitter, or may be recorded for further image processing, by conventional means. Preferably, successive time-lapse images from all mini-photomicroscopes **20** are recorded together on the same recording medium, for example video tape, by the same recording device.

Preferably, control system **60** directs stepping motors to move mini-photomicroscopes **20** up and down continuously, over an appropriate range of distances, to focus continuously on different focal imaging planes within the biological samples. In this way, time lapse 3D images of the biological samples may be acquired, in the manner of Carlsson, and features that move vertically over time within the biological samples may be monitored.

The means for controlling the environmental parameters such as temperature, humidity and atmospheric composition within incubator **30** are conventional, and therefore are not shown in Figure 2.

The apparatus of Figure 2 may be used to conveniently monitor the development of mammalian embryos in an environment that simulates the conditions,

including total darkness, inside a mammalian womb. For this purpose, incubator **30** is covered with an opaque material to exclude all light. Light source **50** is turned on periodically, only long enough to capture images of the embryos from the solid-state area image sensors of mini-photomicroscopes **20**. While light source **50** is on, mini-photomicroscopes **20** may be moved up and down to focus, from different focal distances, on different focal image planes in the embryos, as described above. Light source **50** may be turned on at regular intervals of on the order of several hours, thereby providing another implementation of time-lapse photomicroscopy of the embryos.

Most preferably, the solid-state area image sensor arrays of mini-photomicroscopes **20** are long integration time CCD arrays, because of their ability to acquire images at very low levels of light intensities. If these sensitive detectors are used, optic fiber cables **52** may be dispensed with, and a light source **50** of sufficiently low intensity not to disturb the embryos may be located outside incubator **30** but within the opaque material

Figure 3 shows an alternative configuration of mini-photomicroscopes **20** within an incubator **30**. In this case, the transparent holder of the biological sample is a capillary tube **36** of square cross section, seen end-on in Figure 3. Mini-photomicroscopes **20** are focused on the same point in the biological sample, the intersection point **27** of optical axes **26** of mini-photomicroscopes **20**. As in the configuration of Figure 2, fiber optic cables **52** are provided for shining light into capillary tube **36** from the sides opposite mini-photomicroscopes **20**. For simplicity, neither mountings **40** nor the means for holding capillary tube **36** are shown in figure

3. In the example of Figure 3, optical axes **26** intersect at a right angle. However, the scope of the present invention also includes capillary tubes of any suitable polygonal cross section: to minimize optical distortion, mini-photomicroscopes **20** are mounted with respect to the capillary tube so that optical axes **26** are perpendicular to the walls of the capillary tube, optical axes **26** then intersecting at whatever angle corresponds to that geometric arrangement.

Figure 4 is a schematic diagram of an alternative embodiment of the mini-photomicroscope of Figure 1B. In mini-photomicroscope **20'** of Figure 4, adapter **22** is provided with a mirror **70** intersected by optical axis **26**, and a port **74** through which light is directed at mirror **70**, for example via a fiber optic cable **72**. This light is reflected by mirror **70** through the lens of objective **24**, and thereby focused on a focal image plane in a biological sample. If the light introduced via fiber optic cable **72** is visible light, this configuration enables reflection photomicroscopy. If the light introduced via fiber optic cable **72** is ultraviolet light, this configuration enables epifluorescence microscopy. If the light introduced via fiber optic cable **72** is infrared light, this configuration enables infrared microscopy, particularly Fourier Transform infrared microscopy.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made.

WHAT IS CLAIMED IS:

1. A method for monitoring the development of a biological sample in an incubator, comprising the steps of:

(a) providing, inside said incubator, at least two mini-microscopes, each of said mini-microscopes including:

(i) a microscope objective, and

(ii) a solid-state area image sensor array, optically coupled to said microscope objective;

(b) placing the biological sample in a transparent holder inside said incubator; and

(c) focusing each of said at least two mini-microscopes on a focal image plane, at a focal distance from said mini-microscope, in the biological sample.

2. The method of claim 1, wherein said solid-state area image sensor array is a charge coupled device array.

3. The method of claim 1, further comprising the step of: for at least one of said at least two mini-microscopes:

(d) providing a light source on a side of said transparent holder opposite to said mini-microscope, thereby enabling transmission microscopy.

4. The method of claim 3, further comprising the step of: for said at least one mini-microscope:

(e) alternately activating and deactivating said light source.

5. The method of claim 4, further comprising the step of: for said at least one mini-microscope:

(f) recording an image from said solid-state area image sensor array, thereby implementing time lapse photography of said biological sample.

6. The method of claim 3, wherein said light source includes a fiber optic cable.

7. The method of claim 3, further comprising the step of: for said at least one mini-microscope:

(e) displaying an image from said solid-state area image sensor array.

8. The method of claim 1, further comprising the step of: for at least one of said at least two mini-microscopes:

(d) providing mechanism for focusing incident radiation through said microscope objective on said focal image plane.

9. The method of claim 8, wherein said incident radiation is ultraviolet light, thereby enabling epifluorescence microscopy.

10. The method of claim 8, wherein said incident radiation is visible light, thereby enabling reflection microscopy.

11. The method of claim 8, wherein said incident radiation is infrared light.

12. The method of claim 1, wherein said transparent holder is a Petri dish.

13. The method of claim 12, wherein each of said at least two mini-microscopes is focused on a different portion of the biological sample.

14. The method of claim 1, wherein two of said two mini-microscopes are focused on one portion of the biological sample.

15. The method of claim 14, wherein each of said two mini-microscopes has an optical axis, said two optical axes intersecting within said one portion of the biological sample.

16. The method of claim 15, wherein said transparent holder is a capillary tube having a polygonal cross section.

17. The method of claim 16, wherein said polygon is a square, and wherein said two optical axes intersect at a right angle.

18. The method of claim 1, further comprising the steps of: for at least one of said at least two mini-microscopes:

(d) varying said focal distance; and

(e) recording a plurality of images from said solid-state area image sensor array as said focal distance is varied, thereby providing a three-dimensional record of said biological sample.

19. An apparatus for monitoring the development of a biological sample, comprising:

(a) an incubator;

(b) a transparent holder mounted within said incubator; and

(c) at least two mini-microscopes, mounted relative to said transparent holder within said incubator so that, when the biological sample is placed in the transparent holder, each of said at least two mini-microscopes may be focused on a focal image plane within the biological sample.

20. The apparatus of claim 19, wherein each of said at least two mini-microscopes includes:

(i) a microscope objective, and

(ii) a solid-state area image sensor array, optically coupled to said microscope objective.

21. The apparatus of claim 20, wherein said solid-state area image sensor array is a charge coupled device array.

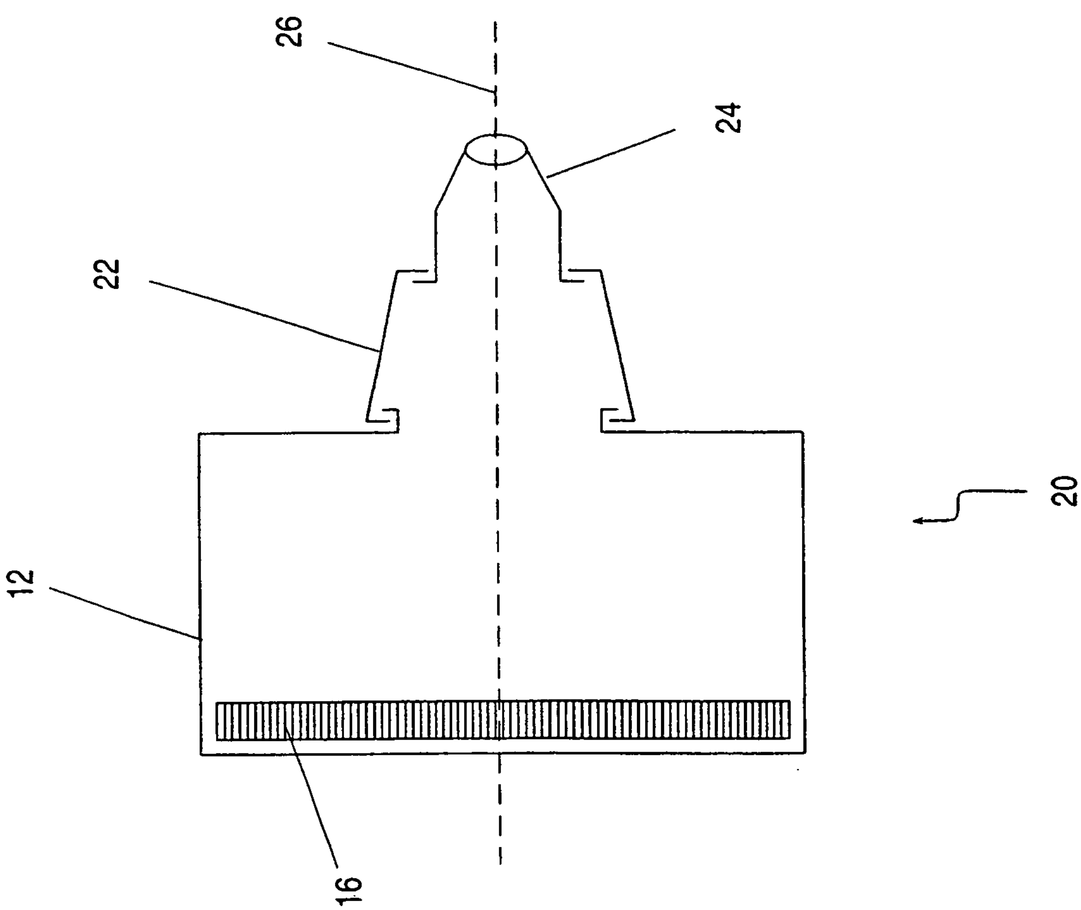

FIG. 1B

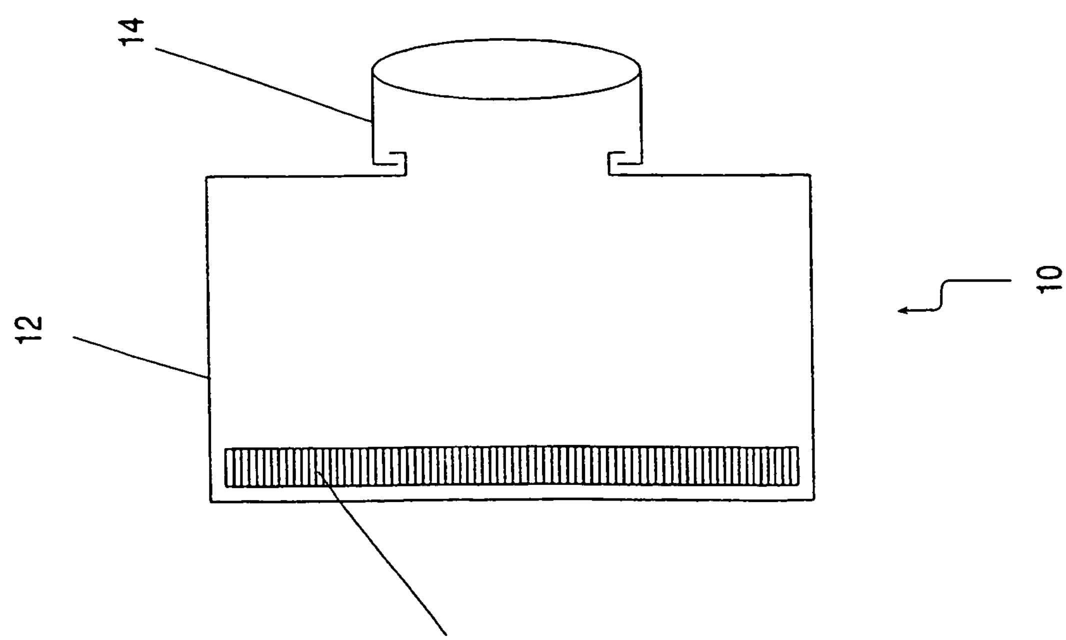

FIG. 1A

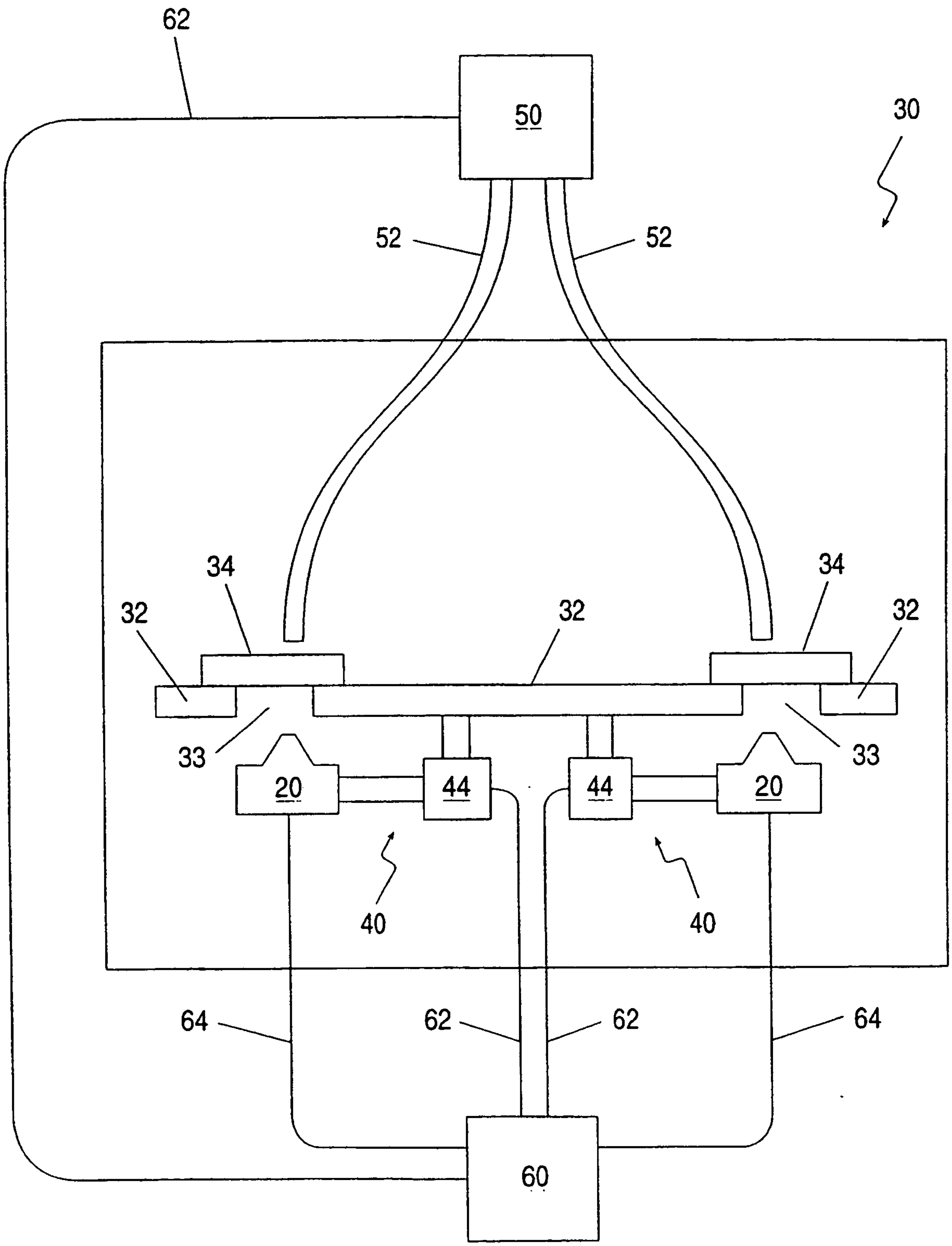

FIG. 2

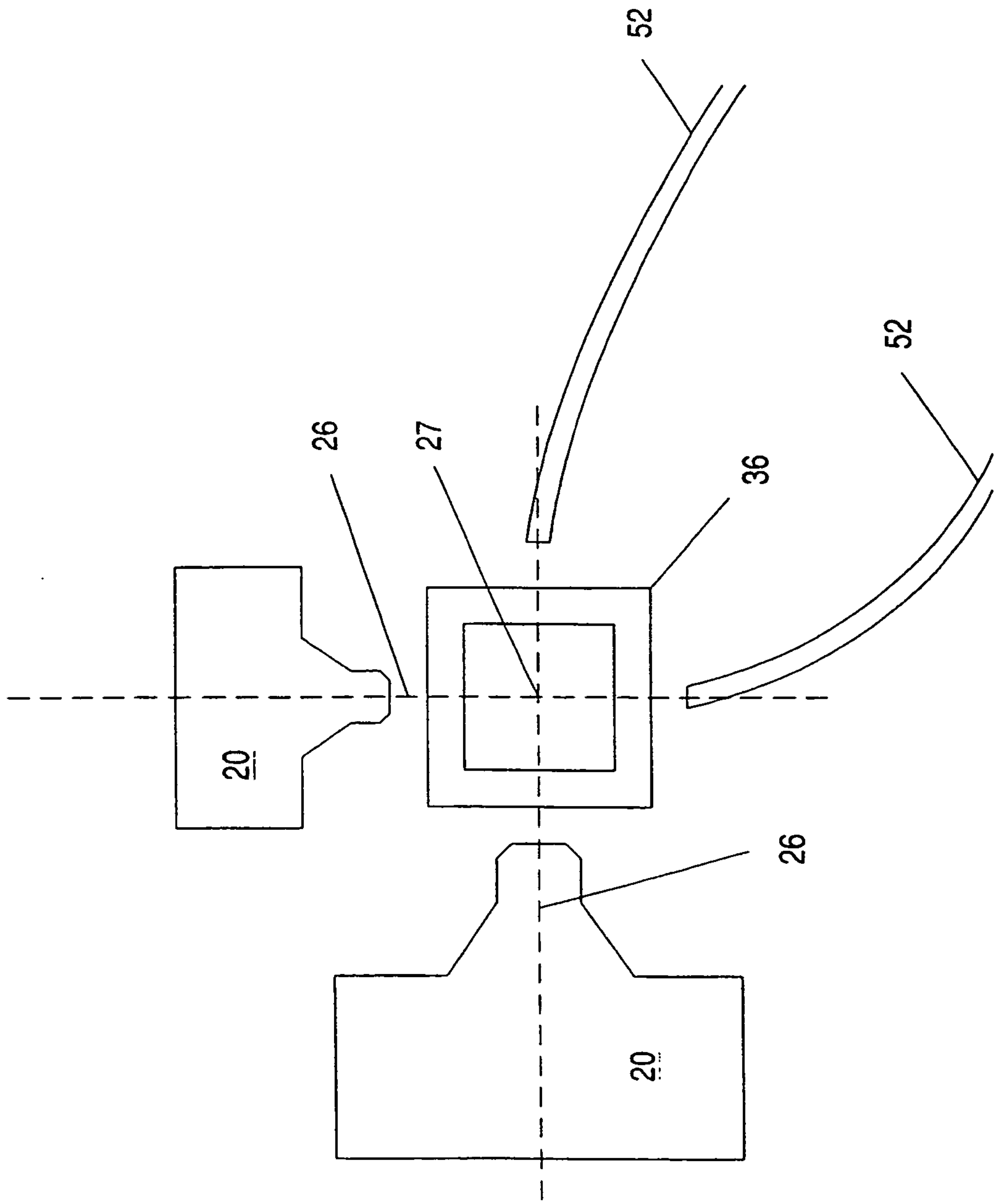

FIG. 3

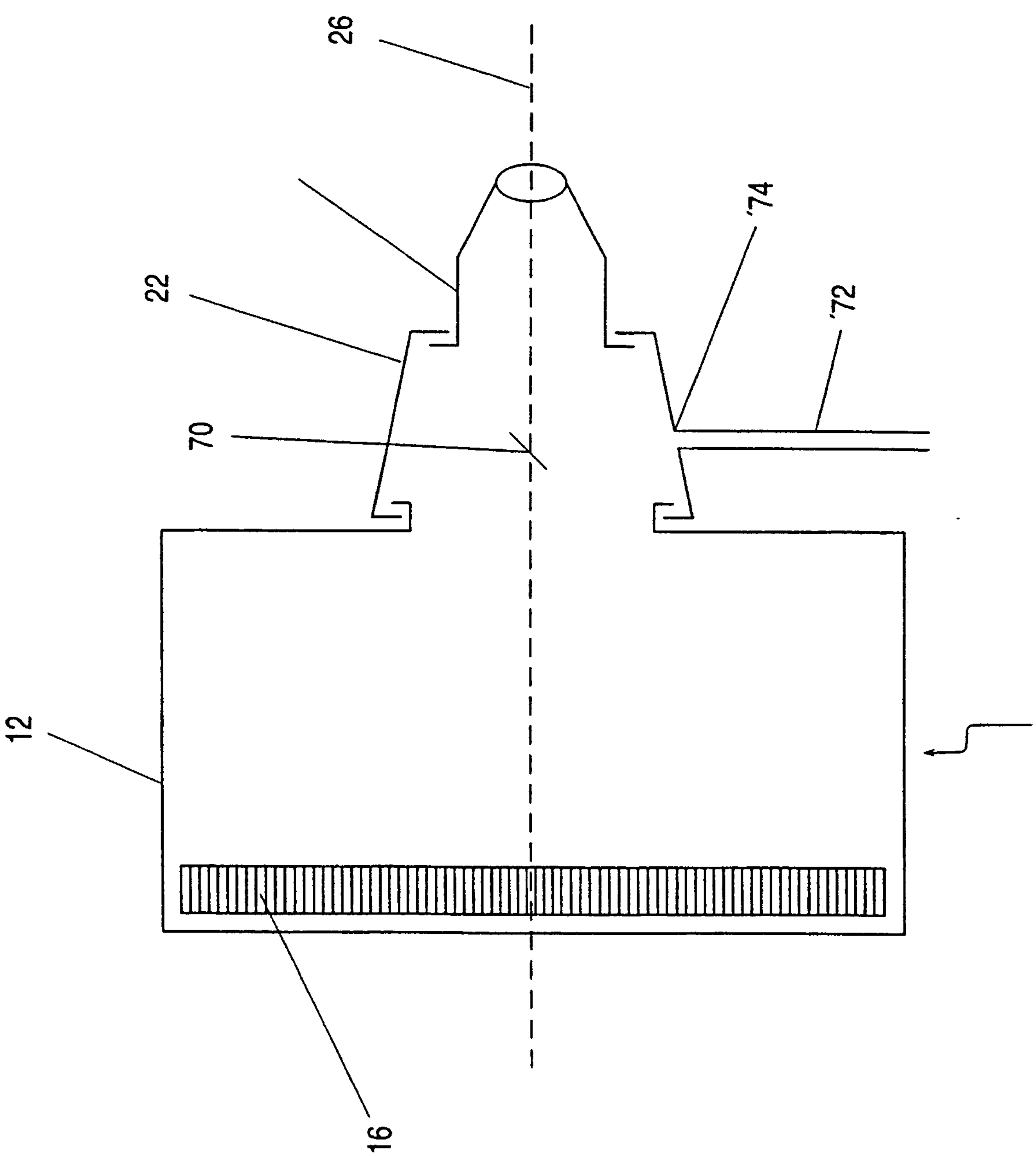

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL98/00009

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :H04N 7/18
US CL :348/80
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 348/42, 46, 47, 61, 79, 80

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Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,397,709 A (BERNDT) 14 March 1995, col.9, lines 8-17 and Figure 7.	1-21
Y	US 5,109,276 A (NUDELMAN ET AL) 28 April 1992, col.7, line 62, to col.8, line 17.	1-21
Y	US 5,465,114 A (MIYAMOTO) 07 November 1995, col.8, lines 47-61, col.9, lines 2-4, and Figure 1.	3-7, 12, 13
Y	US 5,541,081 A (HARDY ET AL) 30 July 1996, col.11, lines 52-57.	9, 11
Y	US 4,902,132 A (MURPHY, JR. ET AL) 20 February 1990, col.1, lines 38-42.	16, 17
A	US 5,307,161 A (MIYAMOTO) 26 April 1994, Figure 3.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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